Synthesis of Methoxy Benzodipyrrole

Presented by Aditi Ghatak-Roy

In partial fulfillment of the requirements for graduation with the Dean's Scholars Honors Degree in Biology

_____ _____
(Name) Date
Supervising Professor

_____ _____
(Name) Date
Co-Supervising Professor

_____ _____
(Name) Date
Honor's Advisor in the Department of Biological Sciences

Abstract

Benzodipyrroles are aromatic heterocyclic that have elicited considerable interest in various fields of chemistry, for example in the development of ionophores for oxanions,[2] antitumor drugs,[3] and as semiconductive materials.[8,9] The use of benzodipyrroles has been widely explored in the treatment of several kinds of infectious diseases.[3] In addition, these molecules have been seen to serve as effective precursors to porphycenes, which are potential photosensitizers for photodynamic therapy. The study of a promising class of photosensitizers, called dibenzoporphycenes, has been limited due to their difficult synthesis and low solubility.[4] Hence, the synthesis of new benzodipyrrole for the synthesis of more soluble benzoporphycenes is needed. This thesis describes the synthesis and characterization of the more soluble dimethoxybenzodipyrrole. The synthesis of methoxy benzodipyrroles was straightforward, clean and had a workable level of solubility in organic solvents. However, dimethoxy benzodipyrrole was obtained in modest yields (~ 8%). This compound appears to be a viable precursor for future synthesis of dibenzoporphycenes.

Introduction

Benzodipyrroles are heterocyclic structures constituted of two pyrroles linked by a double bond on one the β-positions and can occur in five possible isomers (cf. Figure 1). These molecules were first synthesized in gram scale by Berlin *et al. via* the reduction of bis-enamines.[1] These aromatic molecules have been attracted continued interest for their potential applications in various fields, including materials (i.e., electroconductive polymers),[1] sensors (i.e., the creation of anion-selective electrodes),[2] antitumor agents (i.e., antitumor antibiotic CC-1065),[3] and photodynamic therapy (PDT) (i.e., as part of dibenzoporphycenes).[4,2] Hence the synthesis of new benzodipyrroles has been the subject of synthetic efforts in recent years.

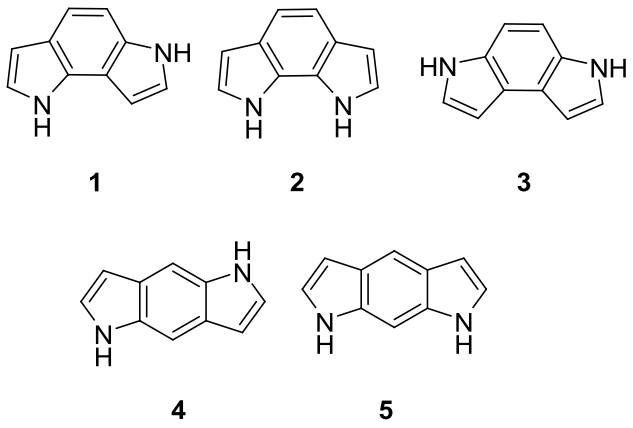
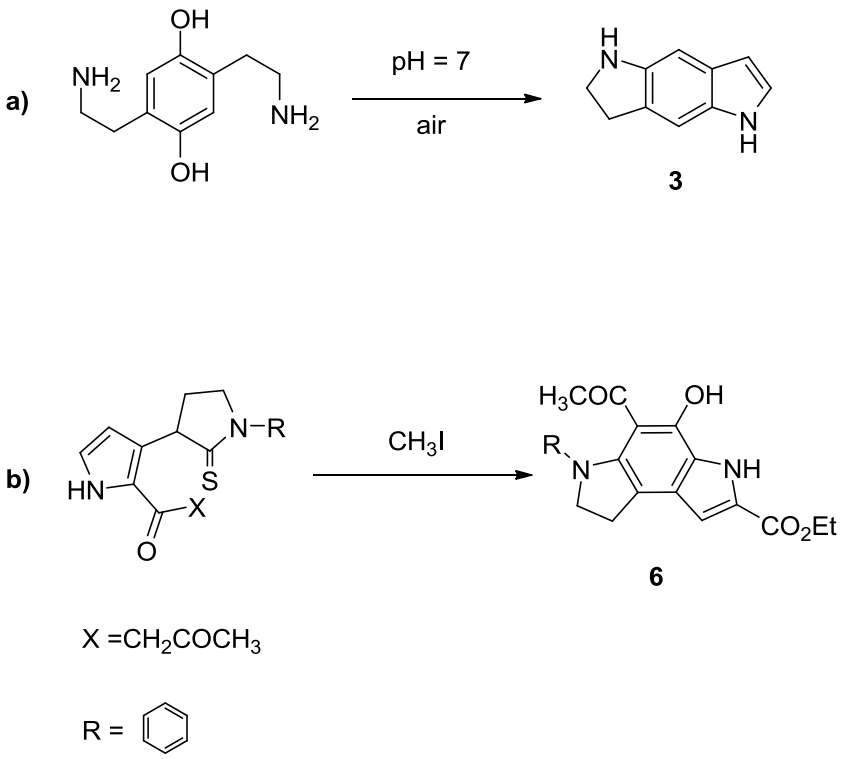


Figure 1: Five possible isomers of benzodipyrroles (**1**-**5**).

In 1973, Baxter and coworkers developed the synthesis of benzodipyrroles *via* intermolecular reactions between the quinol **6** and aliphatic primary and secondary amines in presence of air and at pH 7 led to the formation of benzodipyrroles **3** (cf. Scheme 1).[5] However, this synthetic approach were inefficient and extremely laborious. Later in 1985, Sundberg *et al.* synthesized benzo[1,2-*b*:4,3-*b'*]dipyrroles by intramolecular condensation of 3-(3-pyrrolyl)thiopyrrolidones (cf. Scheme 1).[3] In the initial study, the thiopyrrolidone nitrogen was substituted by a benzyl group. However, the facile dehydrogenation of the indole ring by the usual debenzylation catalysts proved to be a major problem.



Scheme 1: a) Synthesis of benzodipyrrole **3** using quinol in presence of air and pH 7.[5] b) Synthesis of benzodipyrrole **6** using 3-(3-pyrrolyl)thiopyrrolidones as starting materials.[3]

Benzodipyrroles as Building Blocks of Sensitizers for Photodynamic Therapy

Photodynamic therapy (PDT) has been successfully used against precancerous and cancerous masses, by using photosensitizing drugs. The treatment uses a few key components: a localized photosensitizer, or a molecule that is light sensitive, oxygen, and the administration of a specific wavelength of light (cf. Figure 2).[7] Light activation promotes radicalization of oxygen species, which causes the destruction of cells only in the particular areas of tissue that have been exposed to light.[8] In 1903, von Tappeiner and Jesionek, used PDT to treat skin tumors with topically applied eosin, a fluorescent red dye, and white light.[9] Other potential application for PDT is antimicrobial PDT (ADPT), which has been proposed as a possible alternative to antibiotic treatment for localized infections.[10] In this technique, photosensitized processes promoted by irradiation of visible light inactivate microbial pathogens. However, Gram-negative bacteria were resistant to the photosensitizer interaction. This behavior was attributed to a hindrance of photoactivated sensitizers by the cytoplasmic

membrane.[9] As Gram-negative bacteria are often responsible for severe pathological situations, new perspectives in the field have attempted to create porphycene conjugates.

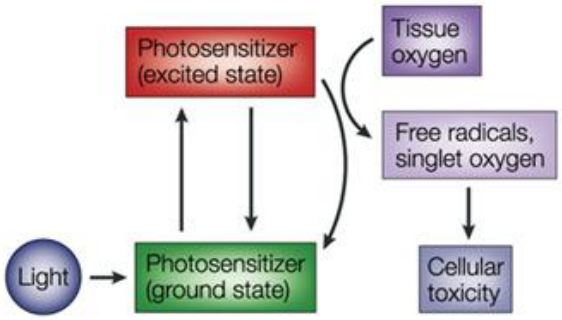
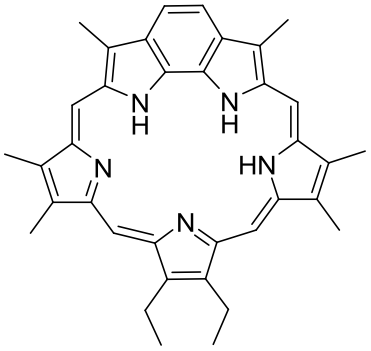
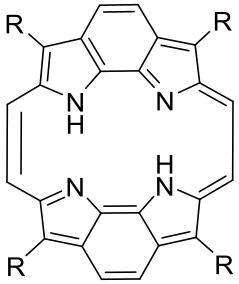


Figure 2: Schematic of photodynamic therapy process.[7]

In the quest for the improvement of PDT efficacy more selective and potent sensitizers are being developed continually. Among the molecules proposed as potential sensitizers are sapphyrins and porphycenes. In particular, the study of these macrocycles that are constituted by benzodipyrroles have been of great interest in recent years.[6, 7] In 2005, Lee and coworkers reported the synthesis of a benzodipyrrole-derived sapphyrin **7** which is constituted by a benzodipyrrole subunit and tripyrromethane dicarboxylic acid (cf. Figure 3). This oligopyrrolic macrocycle exhibited red-shifter absorptions bands, in which the use of the benzodipyrrole unit could provide fine tuning of the electronic properties of the macrocycle.[7, 8] On the other hand, tetraalkylated dibenzoporphycenes, such as tetra-*tert*-butyl dibenzoporphycene **8** and tetramethyldibenzoporphycene **9** (c.f. Figure 3), have been synthesized for potential use in PDT applications.[1, 4] Unfortunately, these macrocycles have been found to be highly insoluble in organic solvents and water, which makes their study extremely challenging.



7



8, R = CH_3
9, R = *t*-Bu

Figure 3: Benzodipyrrole-derived sapphyrin **7**,[6] and tetralkylated dibenzoporphycenes **8** and **9**.[9]

Biological Properties of Benzodipyrroles

Since the isolation of benzodipyrroles as part of a study of phosphodiesterase inhibitors (PDE I and PDE II) by Umezawa and coworkers,[10] the synthesis and biological properties of benzodipyrroles have been widely studied in the development of antitumor drugs.[3] Phosphodiesterase inhibitors PDE I and PDE II, which contain the benzo[1,2-b:4,3-b']dipyrrole ring. The substitution pattern of the phosphodiesterase was also found in the potent antitumor antibiotic CC-1065 produced by *Streptomyces zelensis* NRRL 11,183 (c.f. Figure 4).[3] This compound was isolated by researchers at The Upjohn Company by Hurley and coworkers in 1986 from soil cultures. The X-ray diffraction analysis revealed that CC-1065 is comprised of three benzodipyrrole subunits (subunits A, B, and C) connected by amide bonds.[3] In this report, CC-1065 displayed both cytotoxic activity against L1210 cells in culture, and *in vivo* activity against P388 leukemia in mice.

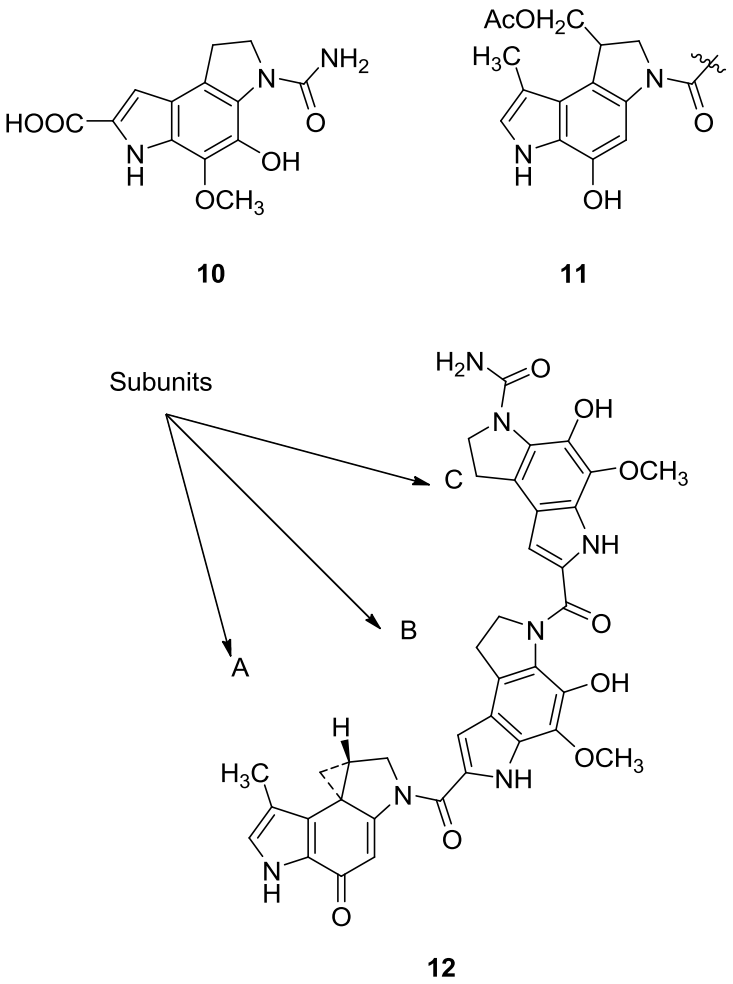


Figure 4: Photodiesterase inhibitor PDE I **10**, and acetic acid degradation product of CC-1065 **11**.[9] Structure of CC-1065 **12**.

CC-1065 has several important chemical features, which are crucial in its biological role. It is a structurally and biosynthetically unique antitumor antibiotic that is believed to target DNA by bind covalently to DNA through N3 of adenine. In a study by Li *et al.*, CC-1065 potency was measured as the degree of drug-induced inhibition of L1210 cell growth.[12] A strong reduction in CC-1065 potency occurred upon premixing with calf thymus DNA, indicative of the weak inhibition of thymidine kinase activity by CC-1065. In addition, the right-handed twist along the length of the molecule suggests binding within one of the grooves of DNA. Several hydrophobic and hydrophilic groups are distributed along the concave and convex edges of the drug molecule, which is important in aiding binding of CC-1065 in the minor groove of DNA (cf. Figure 4 Compound **10**). Another observed attribute of CC-1065 was its effects on cellular macromolecule synthesis, examined in L1210 cells *in*

vitro where DNA synthesis was greatly inhibited. Experimental data showed that CC-1065 was able to interact with the DNA template, thus inhibiting normal DNA polymerase-α activity.

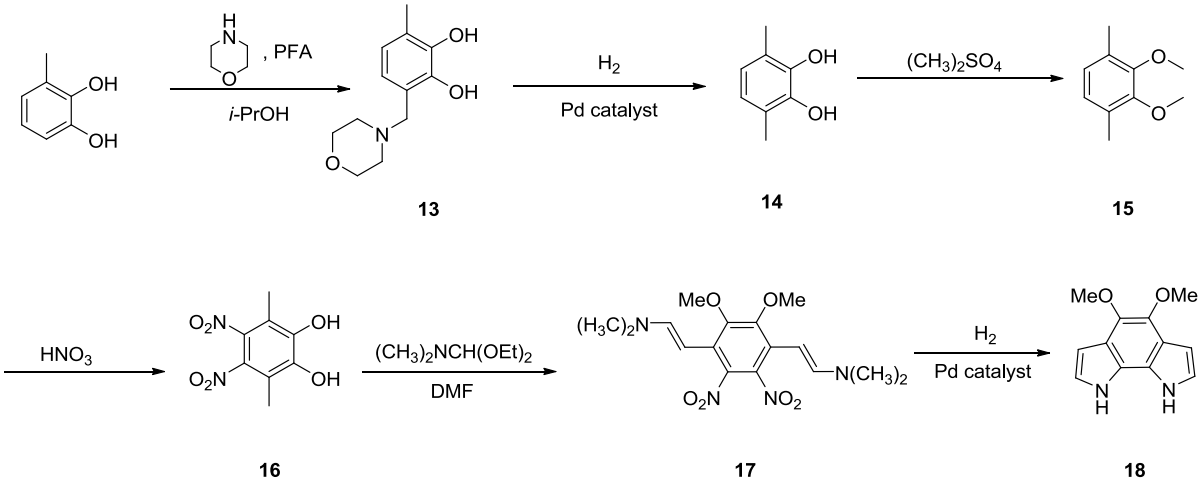
The antitumor activity of CC-1065 was tested using *in vitro* transplantable human tumor cloning assays by Bhuyan *et al.*,[13] It was found that within 1 hour of exposure to 0.1 ng/ml of the drug, there was a 70% decrease in tumor-colony forming units (T-CFU) compared to control plated in 1/9 tumors from lung cancer patients, ½ pancreatic tumors, 1/1 small bowel tumor, and ½ adenocarcinomas of unknown origin. Further decreases (>50%) were seen in breast, ovarian, pancreatic, stomach and testicular cancers. Not as significant decreased (< 50%) were seen in patients with melanoma, leukemia, esophageal cancer, colon cancer, or sarcoma.

With these developments in benzodipyrrole chemistry in mind, the development of more soluble benzodipyrroles was explored. These can serve as a means to develop precursors of dibenzoporphycenes. It is been seen that using pyrrole-based research, modifications to precursors at the *meso*-carbon atoms and/or peripheral β-pyrrole carbon atoms of strongly electron-withdrawing groups can prevent degeneration.[4] Addition of methoxy groups is predicted to increase solubility of this class of molecules.

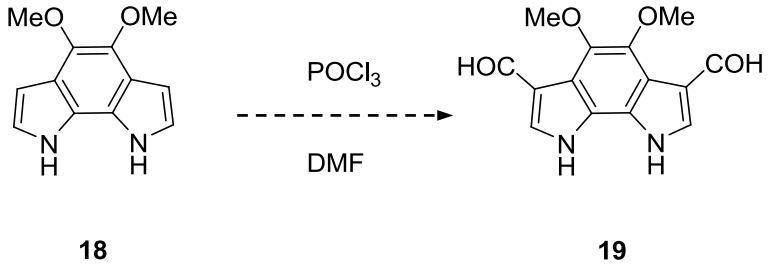
Discussion and Results

The synthesis of dimethoxybenzopyrrole **18** is presented in Scheme 2. It starts with the reaction between morpholine, 3-methyl catechol, and formaldehyde, which afforded 3-methyl-6-(morpholine methyl)benzene-1,2-diol **13** in 43.4% yield. 3,6-dimethyl-benzene-1,2-diol **14** was obtained by the reaction of **13** with hydrogen gas and Pt catalyst at 70˚C followed by alkylation of the latter compound with dimethylsulfate to produce 2,3-demethoxy-1,4-dimethylbenzene **15**, in 88.7% yield. Subsequent nitration of **15** with concentrated nitric acid (70%) afforded 1,2-demethoxy-3,6-dimethyl-4,5-dinitrobenzene **16** in 36.7% yield. Dimethoxybisenamine **17** was obtained in 59.0 yield by reacting **16** with dimethylformamide diethylacetate under N_2 atmosphere. Finally, the formation of dimethoxybenzodipyrrole **18** was carried out under hydrogen gas (1 atm) using Pt catalyst to obtain 7.6% of the desired product. In an effort to synthesize a tetraformylated benzodipyrrole **19** that could be used as precursor for future synthesis of dibenzoporphycene, formylation of **18** was carried out by reacting $POCl_3$ in DMF (cf. Scheme 3). Unfortunately,

the tetraformylated derivative of compound **18** could not be isolated. This is attributed to a polymerization of **18** under the conditions employed.



Scheme 2: Synthesis of dimethoxy benzodipyrrole **18**.



Scheme 3: Proposed synthesis of tetraformylated dimethoxy benzodipyrrole **19**.

Conclusion

The synthesis of dimethoxy benzodipyrrole **18** yielded a successful formulation of a novel methoxy benzodipyrrole. This compound was fully characterized by proton and carbon NMR spectroscopy as well as by high-resolution spectrometry. Compound **18** could serve as a precursor for the synthesis of more soluble benzoporphycenes. Attempts to obtain the tetraformylated dimethoxy dibenzodipyrrole **19** that could be used as precursor for the synthesis of a more soluble dibenzoporphycene, were not successful. This was attributed to the polymerization of the benzodipyrrole. In the future, further experimentation with this precursor should be conducted in order to obtain porphycenes that may be developed as

photosensitizers for photodynamic therapy. Before that stage, various benzoporphycenes should be successfully synthesized from methoxy benzodipyrrole and characterized for solubility and sensitization to light.

Experimental Synthesis of Dimethoxy Benzodipyrrole

Prior to use, all glassware was soaked in KOH-saturated isopropyl alcohol for ca. 12 hours, rinsed with water and acetone and thoroughly dried. Dry solvents (dichloromethane, tetrahydofuran, methanol, diethyl ether and acetonitrile) were purified by passage through Vacuum Atmosphere solvent drying towers. All other chemicals were used as purchased. All solutions were stirred magnetically. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian Mercury 400 MHz instrument. High-resolution mass spectra were obtained at the University of Texas at Austin, Department of Chemistry and Biochemistry, Mass Spectrometry Facility.

3-methyl-6-(tetrahydro-2H-pyran-4ylamino) benzene-1,2-diol (13): In a 250 mL round bottom flask, morpholine (9.7 mL, 0.1 mol) , added to 3-methylbenzene-1,2-diol, and paraformaldehyde (3.34 g, 0.1 mol) were dissolved in 70 mL of isopropanol. This mixture was refluxed under vigorous stirring at 75˚C for 2 hours. The solution was allowed to cool to room temperature. A second solution of 3-methyl catechol (14 g, 0.11 mol) in 50 mL of isopropanol was added to the flask. The entire solution was stirred at room temperature for 72 hours. Following this, the resulting crude solid was filtered via vacuum filtration with a Büchner funnel. Recrystallization from ethanol yielded 10.3 g (43.4%). ^{1}H NMR (400 MHz, 296 K, $CDCl_3$): δ (ppm) 6.56, 6.54 (d, (J = 0.02 Hz), 1 H), 6.43, 6.41 (d, (J = 0.02 Hz), 1 H), 3.73 (bs, 3 H), 3.66 (s, 2 H), 2.56 (bs, 3 H), 2.21 (s, 3 H). ^{13}C NMR (100.6 MHz, 296 K, $CDCl_3$): δ (ppm) 143.67, 142.65, 123.50, 121.12, 118.84, 117.81, 66.87, 61.52, 52.87, 15.34. HR-ESI (m/z): 223.1195 (calc. For $[C_{12}H_{17}NO_3]^{+1}$: 223.1208).

3,6-dimethyl catechol (14): Morpholine protected 3-methyl catechol (10.3 g, 48 mmol) was dissolved in 150 mL ethanol in a 500 mL round bottom flask. Palladium over carbon catalyst (3 g, 28.2 mmol) was added and the solution gently stirred and refluxed overnight under hydrogen gas atmosphere. The crude was filtered through a pad of celite using a coarse-porosity fritted filter, followed by repeated washing with methanol until the liquid ran clear.

The solution was transferred to a 150 mL round bottom flask, to which 50 mL of 1% v/v hydrochloric acid was added. Vigorous stirring was applied at room temperature for 3.5 hours. The product was extracted with dichloromethane (3x50 mL) and the organic phase was collected. The organic fractions were dried with sodium sulfate and the solution was evaporated under reduced pressure. The compound was purified by chromatography in silica gel ethyl acetate/hexanes, 1:4) to give 3.86 g (57.9%). %). ^{1}H NMR (400 MHz, 296 K, $CDCl_3$): δ (ppm) 6.59 (s, 1 H), 5.00 (s, 1 H), 2.21 (s, 3 H). ^{13}C NMR (100.6 MHz, 296 K, $CDCl_3$): δ (ppm) 141.67, 121.72, 121.42, 15.31. HR-ESI (m/z): 138.0885 (calc. For $[C_8H_{10}O_2]^{+1}$: 138.0681).

2,3-dimethoxy-1,4-dimethylbenzene (15): 3,6-dimethyl catechol (3.86 g, 28.4 mmol was added to a 250 mL three neck round bottom flask with 39.89 mL 10% w/v sodium hydroxide and 11.32 mL dimethyl sulfate. This solution was refluxed for 1.5 hours at 100˚C. After this time, 8.75 mL 40% w/v sodium hydroxide and 5.15 mL dimethyl sulfate was added to the mixture and refluxed for 0.5 hours more. After this time, 4.37 mL 40% w/v sodium hydroxide and 2.57 mL dimethyl sulfate was added to the flask and the mixture refluxed at the same temperature for 1 hour. The mixture was then cooled at room temperature. The solution was extracted with diethyl ether (2x20 mL). The organic fractions were washed with 20 mL water, 15 mL of 10% w/v sodium hydroxide solution, 20 mL of water, and dried with sodium sulfate. The organic solvent was evaporated to obtain an oil of yield: 4.13 g (88.7%). ^{1}H NMR (400 MHz, 296 K, $CDCl_3$): δ (ppm) 6.78 (s, 4 H), 3.81 (s, 2 H), 2.21 (s, 2 H). ^{13}C NMR (100.6 MHz, 296 K, $CDCl_3$): δ (ppm) 125.87, 125.22, 121.34, 60.53, 60.00, 15.56, 15.31. HR-ESI (m/z): 166.2170 (calc. For $[C_{10}H_{14}O_2]^{+1}$: 166.0994).

1,2-dimethoxy-3,6-dimethyl-4,5-dinitrobenzene (16): Compound **3** (4.13 g, 25.2 mmol) was added to a 250 mL round bottom flask and the mixture was placed in an ice water bath. Nitric acid (34.76 mL) was added dropwise under constant stirring. The ice bath was removed and flask transferred to an oil bath. The solution was heated to 70˚C for 4 hours, and then allowed to cool at 40˚C. The crude material was poured in 100 mL ice and a saturated solution of sodium carbonate was added under stirring. The material was collected by filtration to yield 2.24 g (36.7%). ^{1}H NMR (400 MHz, 296 K, $CDCl_3$): δ (ppm) 7.24 (s, 2 H), 3.88 (s, 6 H), 2.27 (s, 6 H). ^{13}C NMR (100.6 MHz, 296 K, $CDCl_3$): δ (ppm) 153.33, 125.82, 60.66, 11.80. HR-ESI (m/z): 228. 0416 (calc. For $[C_8H_8N_2O_6]^{+1}$: 228.0382).

2,2-(dimethylamino)vinyl-2,3-dimethoxy-5,6-dinitrophenyl)-N,N'-dimethylethenamine (17): In a two neck 250 mL round bottom flask, Compound **4** (2.24 g, 9.2 mmol) and dimethylformamide diethyl acetal (9.51 mL) was dissolved in 20 mL dry dimethylformamide. The system was kept under nitrogen gas atmosphere. The solution was refluxed under argon for 24 hours at 115˚C. The flask was allowed to cool at room temperature, and the remaining solvent evaporated under reduced pressure. The final product was recrystallized from diethyl ether until violet crystals yielded 2.0 g (59.0%). ^{1}H NMR (400 MHz, 296 K, $CDCl_3$): δ (ppm) 7.24, (s, 3 H), 4.76 (d, (J = 0.02 Hz), 2 H), 3.78 (s, 6 H), 2.81 (s, 12 H). ^{13}C NMR (100.6 MHz, 296 K, $CDCl_3$): δ (ppm) 151.33, 146.29, 122.99, 84.22, 59.68, 40.33. HR-ESI (m/z): 366.1510 (calc. For $[C_{16}H_{22}N_4O_6]^{+1}$: 366.1539).

Methoxy benzodipyrrole (18): In a 250 mL round bottom flask, bisenamine (2 g, 5.5 mmol) and Palladium over carbon catalyst (1.7 g) were added in 200 mL of ethyl acetate. The mixture was stirred under hydrogen atmosphere (1 atm) for 18 hours. The mixture was filtered through a celite pad and with ethyl acetate until the solvent ran clear. The organic solvent was evaporated under reduced pressure. The product was purified by chromatography in silica gel (ethyl acetate/hexanes, 1:5) to obtain the final product **6**, yielding 0.09 g (7.6%). ^{1}H NMR (400 MHz, 296 K, $CDCl_3$): δ (ppm) 8.32 (s, 1 H), 7.06 (s, 1 H), 6.70 (s, 1 H), 4.03 (s, 3 H). ^{13}C NMR (100.6 MHz, 296 K, $CDCl_3$): δ (ppm) 138.29, 121.03, 119.66, 118.75, 101.49, 61.45. HR-ESI (m/z): 216.2359 (calc. For $[C_{12}H_{12}N_2O_2]^{+1}$: 216.0899).

Tetramethyl dimethoxy benzodipyrrole (19): In a 100 mL three neck round bottom flask, Compound **18** (0.09 g, 0.42 mmol) was dissolved in dry dimethylformamide (10 mL). The solution was placed under nitrogen gas with constant stirring at room temperature for 15 minutes. The solution was cooled using an ice bath and the system was purged with N_2. Phosphorous oxychloride (0.16 mL, 1.7 mmol) was added dropwise with a syringe under nitrogen atmosphere. The solution was kept in an ice bath and stirred for one hour. The solution was warmed at room temperature and stirred for another 30 minutes. Water (10 mL) and potassium hydroxide (2 M) solution was added dropwise until the pH of the solution became strongly basic. A precipitate should have appeared at this time, but the resulting compound remained soluble in the organic solvent. The organic solvent was evaporated to obtain yellow oil, which could not be characterized. The aqueous fractions were evaporated also; however, no compound **19** was isolated from this phase either.

References

1. Berlin, A.; Bradamante, S.; Ferraccoli, R.; Pagani, G. A.; Sannicolo, F. *J. Chem. Soc., Chem. Commun.* **1987**, 1176-117.
2. Cuartero, M.; Ortuño, J. A. García, M. S.; Sánchez, G.; Más-Montoya, M. Curiel, D. *Talanta* **2011**, 85, 1876-1881.
3. Sundberg, R. J.; Hamilton, G. S.; Laurino, D. P. *J. Org. Chem.* **1998**, 53, 976-983
4. Sánchez-García, D.; Sessler, J. L. *Chemical Society Reviews* **2008**, *37*, 215.
5. Baird, D. B.; Baxter, I,; Cameron, D. W.; Phillips, W. R. *J. Chem Soc. Perkin Trans 1* **1973**, 832-833.
6. Panda, P. K.; Kang, Y.-J.; Lee, C.-H. *Angew. Chem. Int. Ed.* **2005**, 44, 4053-4055.
7. Kusuhara, D.; Mack, J.; Yamada, H.; Okujima, T.; Ono, N.; Kobayashi, N. *Chem. Eur. J.* **2009**, 15, 10060-10069.
8. Vogel, E.; Koecher, M.; Schmickler, H.; Lex, J. *Angew. Chem., Int. Ed. Engli.* **1986**, *25*, 257.
9. Reynolds, V. L.; Mcgovern, J. P.; Hurley, L. H. *The Journal of Antibiotics* **1986**, *39*, 319–334.
10. Enomoto, Y.; Furutani, Y.; Naganawa, H.; Hamada, M.; Takeuchi, T.; Umezawa, H. *Agric. Biol. Chem.* **1978**, 42, 1337
11. Rawal, V. H.; Cava, M. P. *J. Org. Chem.* **1978**, 52, 19-28.
12. Li, L. H.; Swenson, D. H.; Schpok, S. L. F.; Kuentzel, S. L.; Dayton, B. D.; Krueger, W. C. *Cancer Res*. **1982**, 42, 2821-2828.
13. Bhuyan, B. K.; Newell, K. A.; Crampton, S. L.; Von Hoff, **D. D.** ***Cancer Res.*** **1982**, 42, 3532.
14. Wainwright, M. J. *Antimicr. Chemother.* **1998**, *42*, 13–28.

15. Kuzuhara, D.; Mack, J.; Yamada, H.; Okujima, T.; Ono, N.; Kobayashi, N. *Chemistry - A European Journal* **2009**, *15*, 10060–10069.
16. Kadish, K. M.; Smith, K. M.; Guillard, R. *The Porphyrin Handbook*; Academic Press: San Diego, 2000; Vol. 2.
17. Wu, Y.-D.; Chan, K. W. K. *Journal of Molecular Structure: THEOCHEM* **1997**, *398-399*, 325–332.
18. Dolmans, D. E. J. G. J.; Fukumura, D.; Jain, R. K. *Nature Reviews Cancer* **2003**, *3*, 380–387.
19. Ragàs, X.; Sánchez-García, D.; Ruiz-González, R.; Dai, T.; Agut, M.; Hamblin, M. R.; Nonell, S. *Journal of Medicinal Chemistry* **2010**, *53*, 7796–7803.
20. Polo, L.; Segalla, A.; Bertoloni, G.; Jori, G.; Schaffner, K.; Reddi, E. *Journal of Photochemistry and Photobiology B: Biology* **2000**, *59*, 152–158.

Appendix A

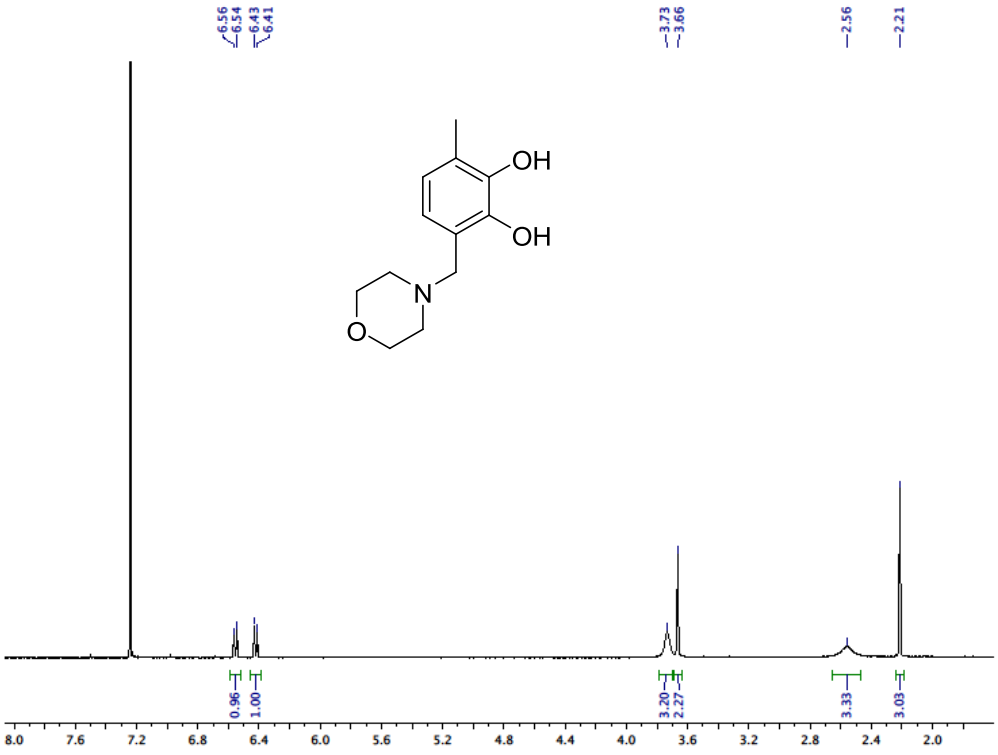


Figure 1: ^{1}H NMR spectrum of Compound **13**.

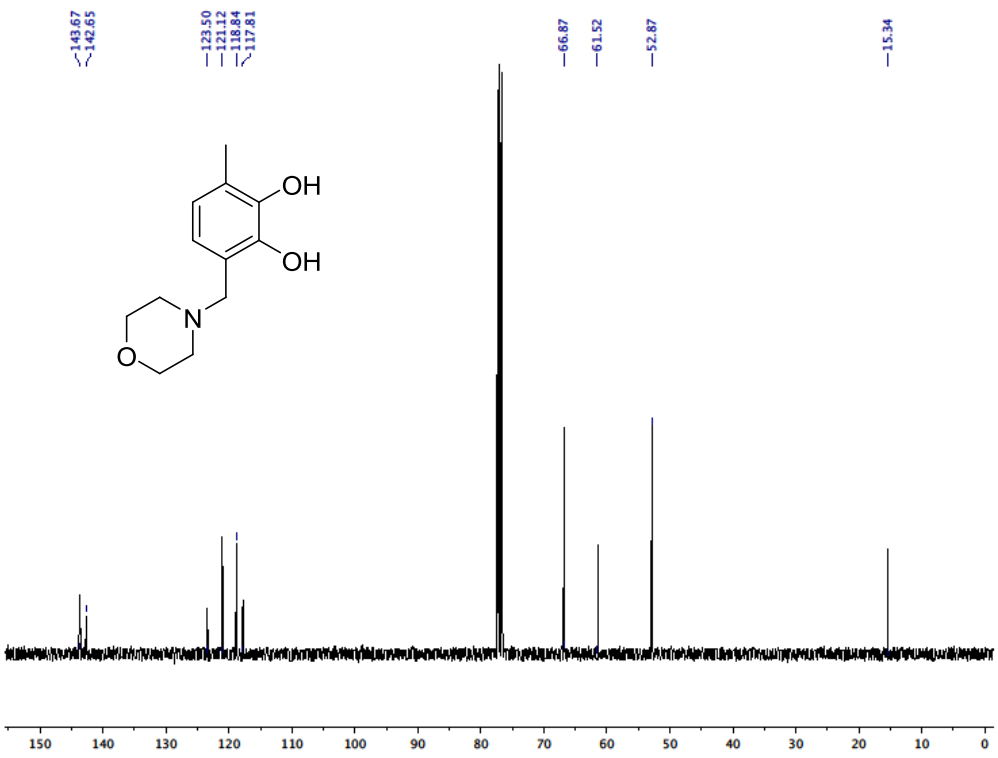


Figure 2: ^{13}C NMR spectrum of Compound **13**.

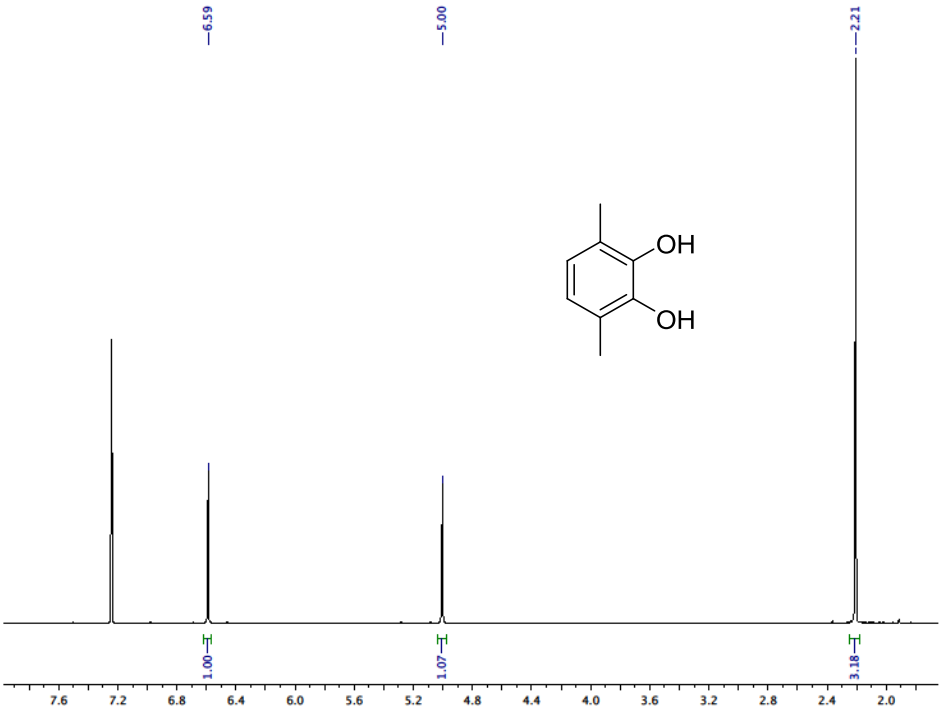


Figure 3: [1]H NMR spectrum of Compound **14**.

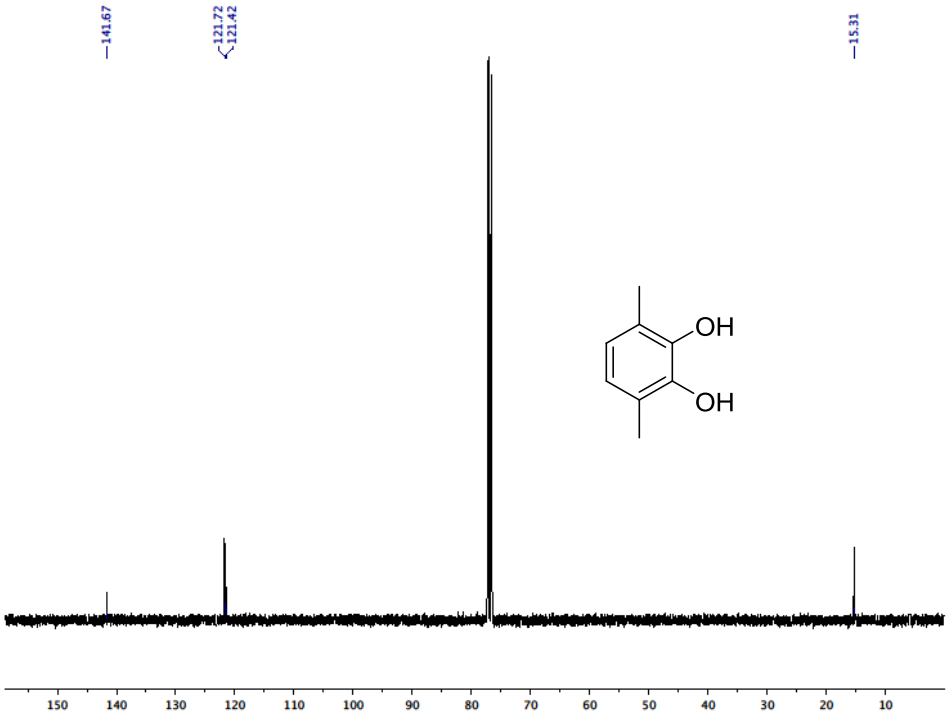


Figure 4: [13] C NMR spectrum of Compound **14**.

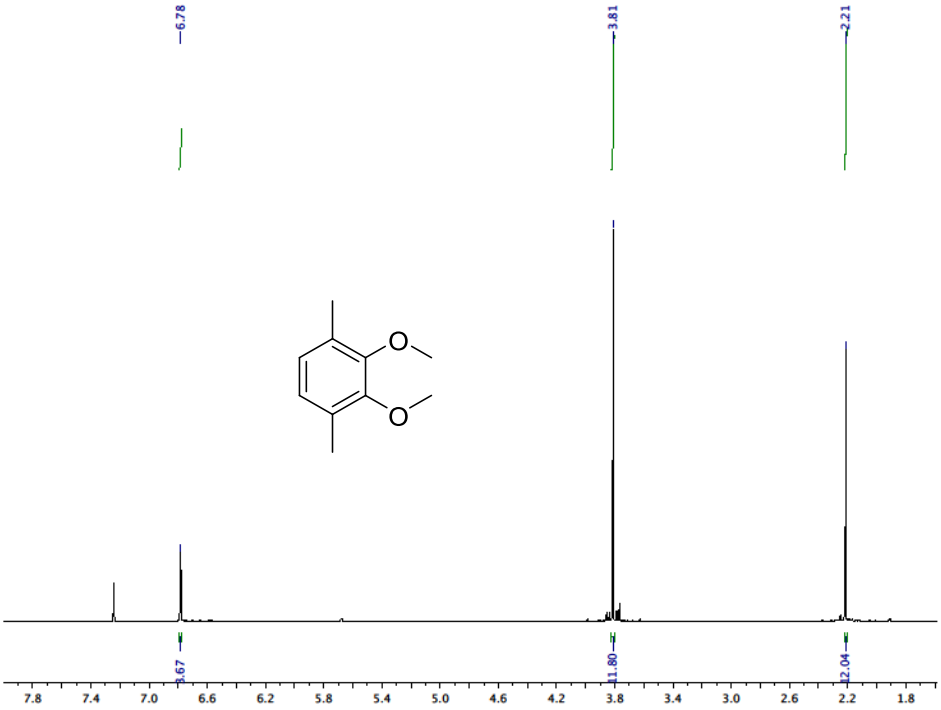


Figure 5: ^{1}H NMR of Compound **15**.

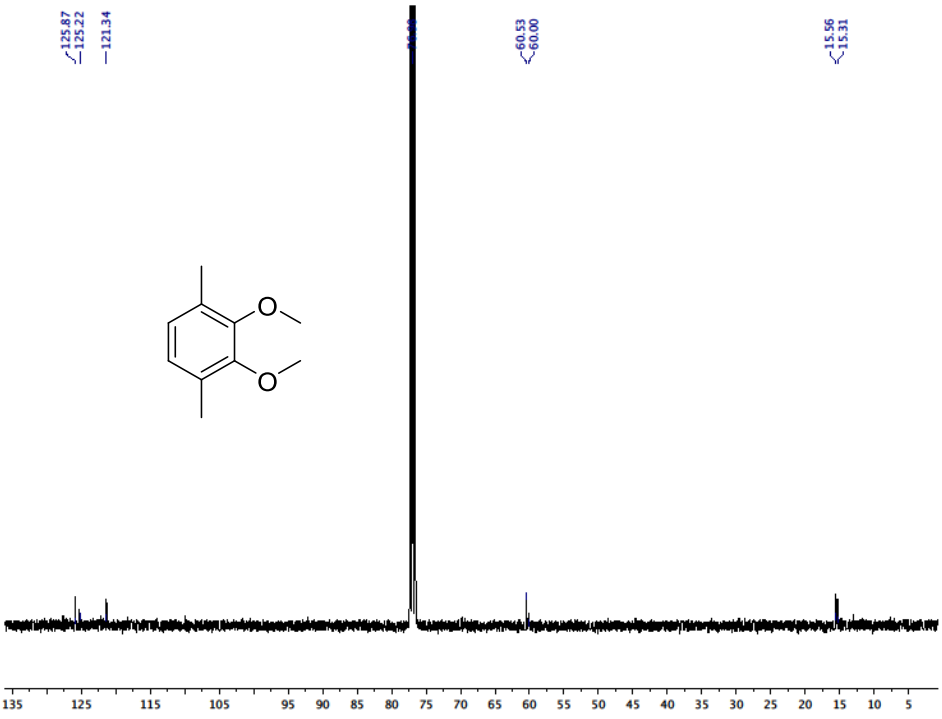


Figure 6: ^{13}C NMR of Compound **15**.

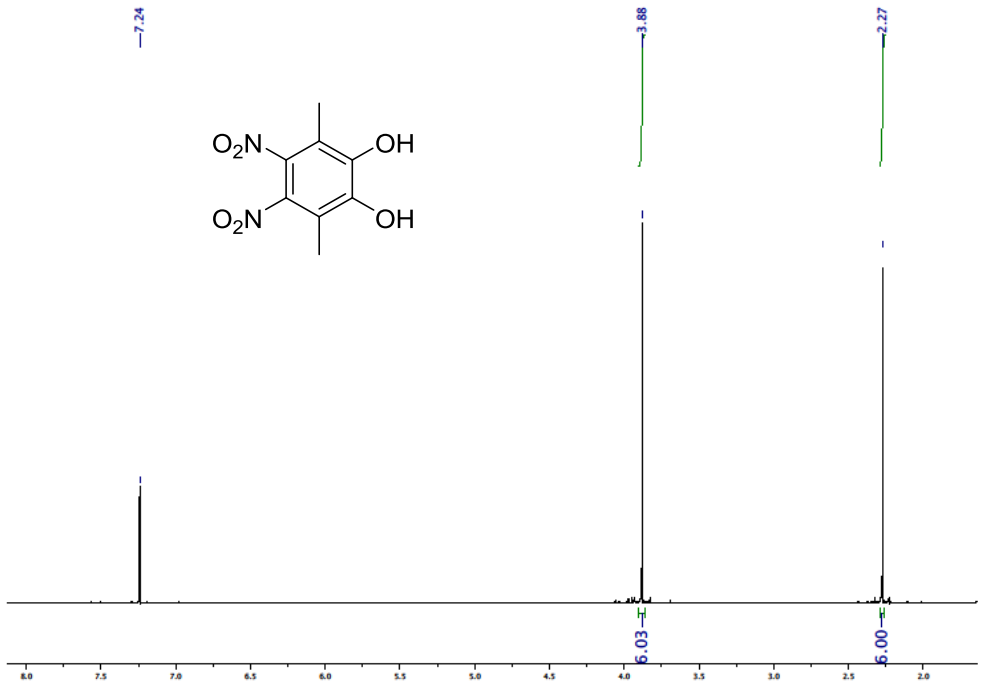


Figure 7: ^{1}H NMR of Compound **16**.

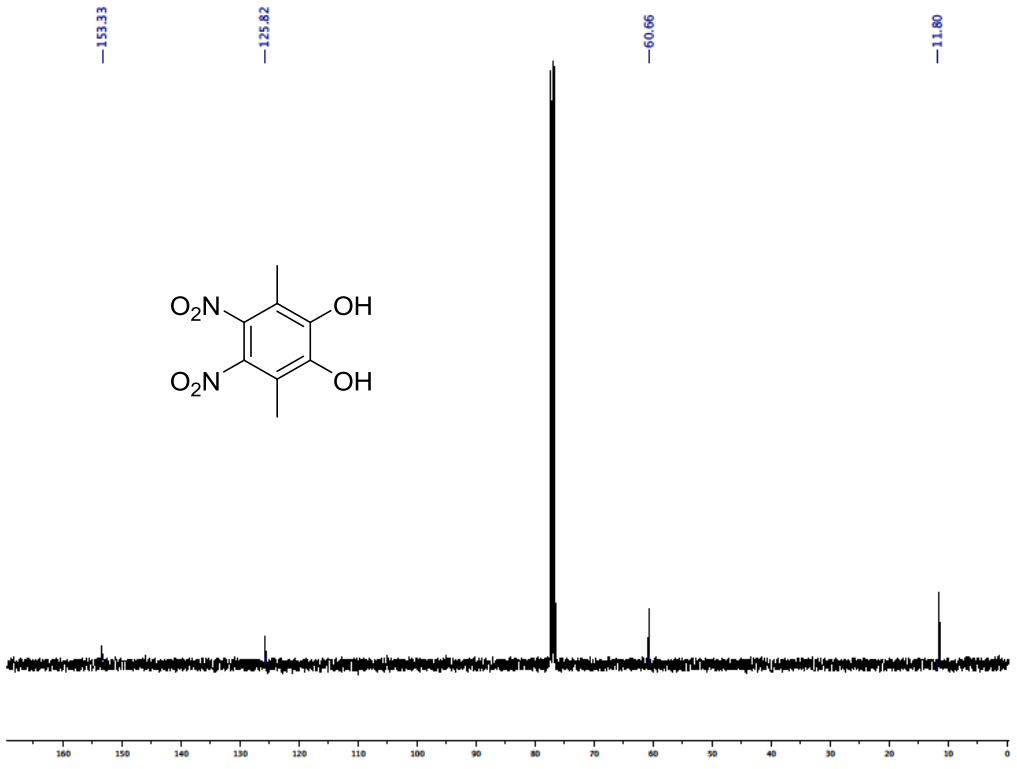


Figure 8: ^{13}C NMR spectrum of Compound **16**.

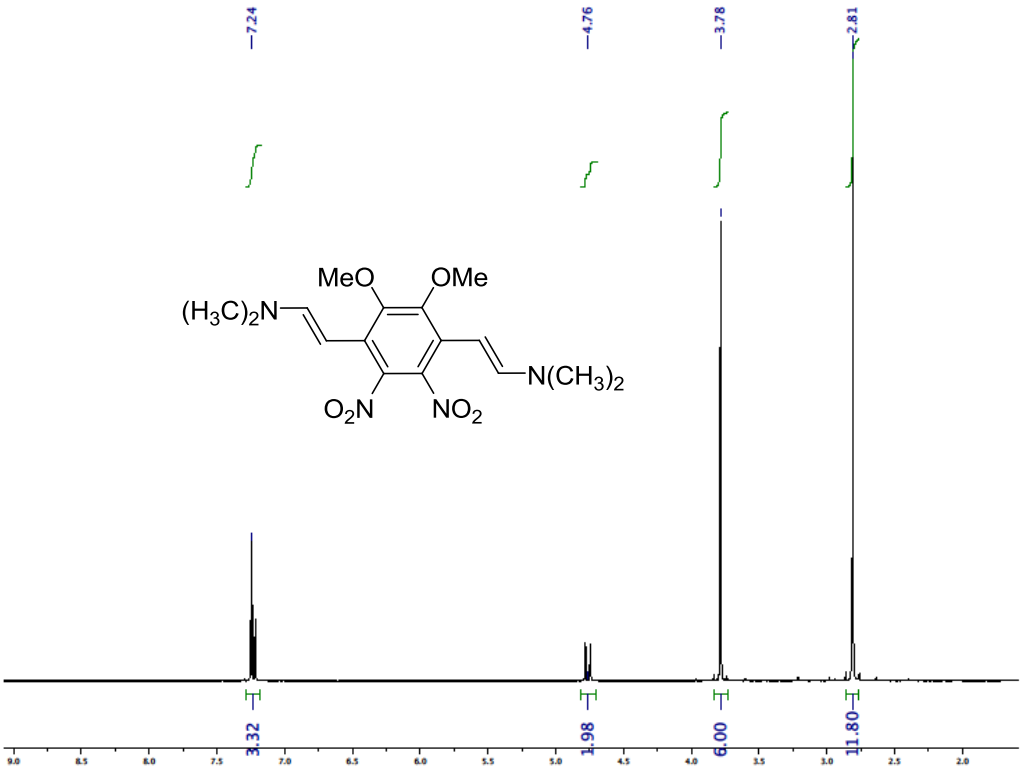


Figure 9: ^{1}H NMR of Compound **17**.

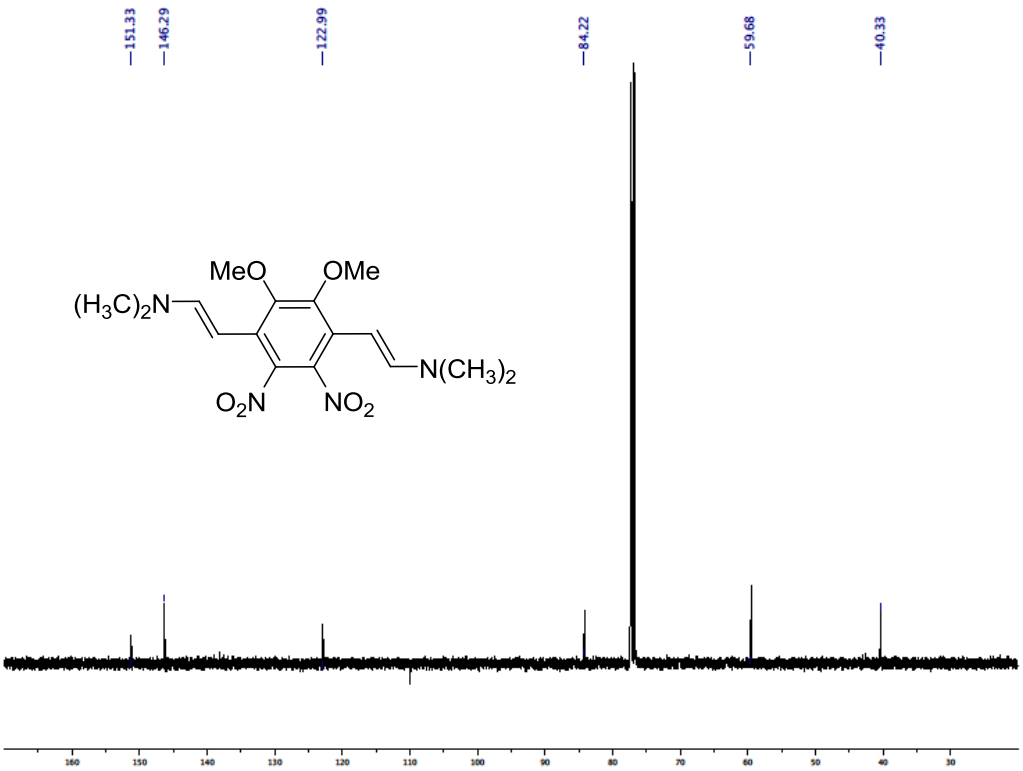


Figure 10: ^{13}C NMR of Compound **17**.

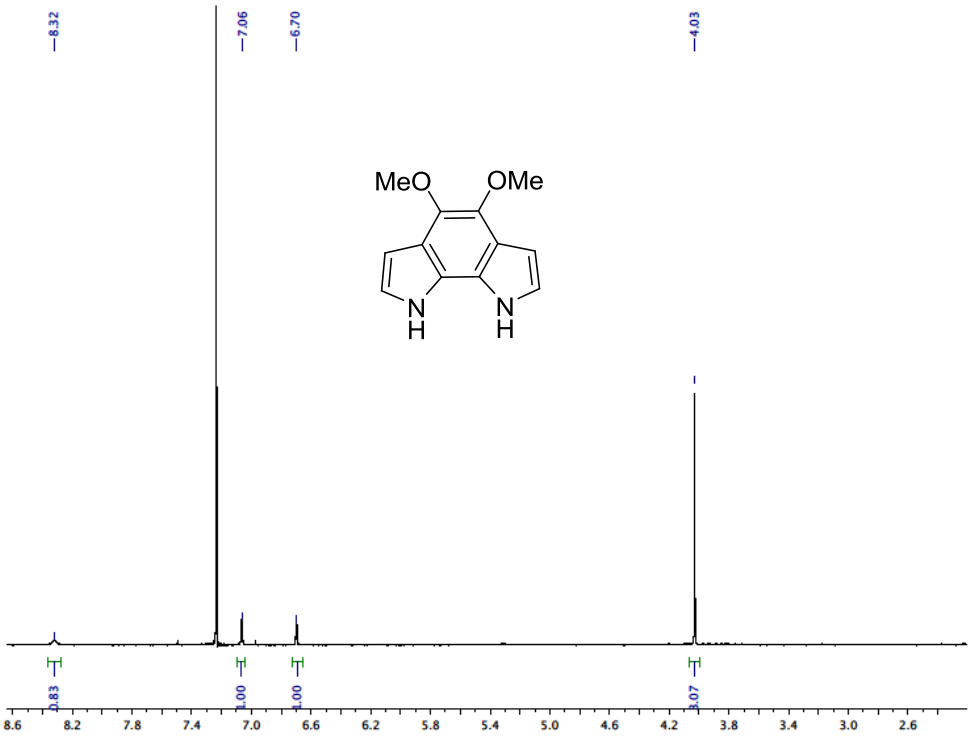


Figure 11: ^{1}H NMR of Compound **18**.

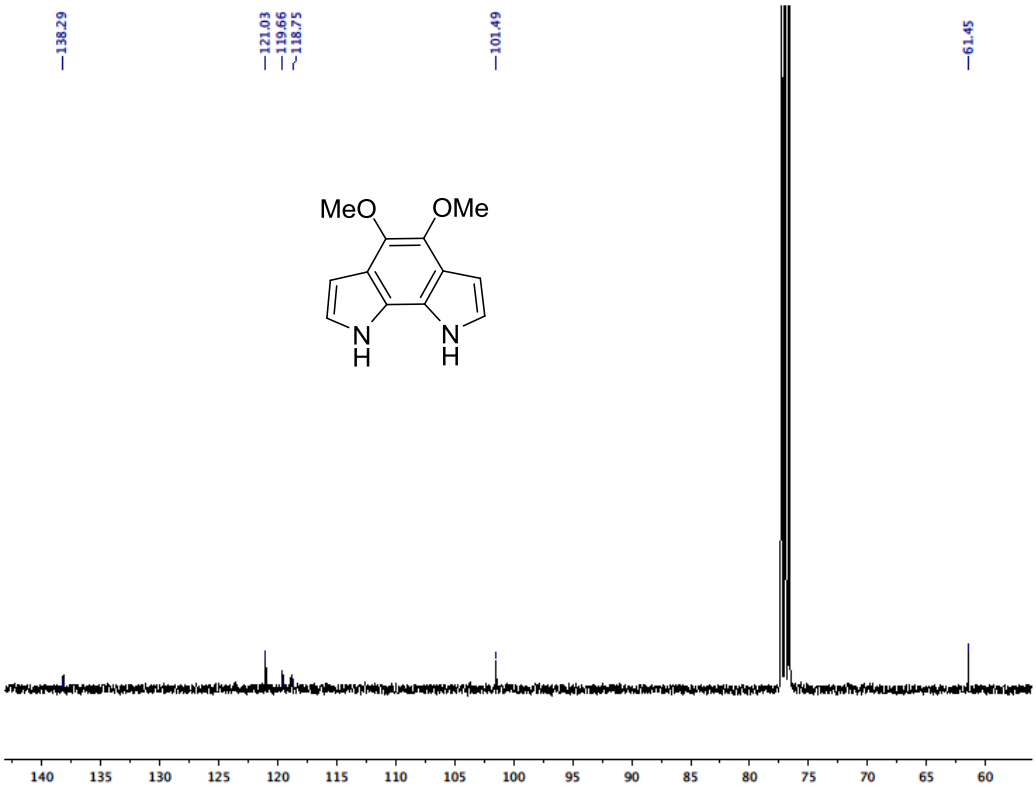


Figure 12: ^{13}C NMR of Compound **18**.